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The energy transfer based fluorescent approach to detect the formation of silica supported phosphatidylcholine and phosphatidylserine containing bilayers

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ABSTRACT

The work introduces the quenching of silica coated Tb(III) complexes by merocyanine 540 (MC540) and copper ions as a tool to reveal the adsorption of phosphatidylcholine (PC) and phosphatidylserine (PS) at various PS:PC ratio onto silica nanoparticles doped with Tb(III) complex. The binding of MC540 with PC-based bilayers and copper ions with PS-based ones are the basis of their use as organic and inorganic probes to sense PS:PC ratio in silica supported mixed bilayers. The enrichment of mixed bilayers by PS results in the displacement of MC540 anions, while it enhances the binding with copper ions. The displacement or binding of probe ions results in the diverse luminescence response of Tb(III)-centred luminescence. The reestablishment of the steady state and time resolved luminescence is observed, when MC540 anions are applied as probes. The use of copper ions as probes results in the opposite quenching effect. The developed route enables to discriminate the formation of the phospholipids bilayers onto silica surface from those in the bulk of solution under various concentration conditions.

1. Introduction

The aggregates of phospholipids (PhLs) have gained a great interest in recent decades as potential drug delivery systems [1–3], as capsules for hazardous luminescent or magnetic nanoparticles [4–7] and for mimicking the cell membrane properties [8–14]. The PhLs bilayers deposited onto silica beads or so-called supported lipid bilayers (SLB) [8–10] are of great importance for both mimicking processes taking part in cell membranes [13] or in growing the blood compatibility of nanomaterial for imaging and drug delivery [11,12]. The widespread application of SLB as mimics of cell membranes prompts great number of works concerning the regularities and conditions of their formation onto silica nanoparticles [14–20]. A number of methods have been applied to probe into the formation of SLB. The electrokinetic potential, surface pressure and adsorption isotherm measurements, atomic force microscopy, Cryo TEM imaging and contact angle measurements are applied to highlight both driving forces and optimal conditions of the SLB formation [14–20]. Nevertheless the development of the procedure to sense the deposition of various PhLs bilayers onto silica beads is rather appealing task. The present work is inspired by an idea to apply donor → acceptor energy transfer as a basis of novel methodological approach to sense a formation of SLBs and to get deeper insight into their binding and inclusion capacities. Our previous works highlight the effect of the adsorption and aggregation of cationic surfactants at the silica/water interface on the energy transfer from Tb(III) complexes coated by silica to the quenching molecules [21,22]. This procedure is based on the switching from the significant quenching of Tb(III)-centred luminescence when quenching molecules are located at the interface of Tb(III)-doped silica nanoparticles (SNs) to the reestablishment of the luminescence through the displacement of quenching molecules from the interface to the bulk of solution. The present work introduces Tb(III)-doped luminescent SNs as luminescent templates for the adsorption of PhLs and the energy transfer from Tb(III) to energy acceptors at the silica/water interface as the tool to reveal the deposition of PhLs bilayers onto SNs. The silica supported mixed bilayers based on zwitter-ionic phosphatidylcholine (PC, Scheme 1) and anionic phosphatidylserine (PS, Scheme 1) are of particular importance due to the mimicking of the cell apoptosis [23,24]. The works [16,17,25,26] highlight the enhanced affinity of cationic and zwitter-ionic phospholipids to negatively charged SNs. The choice of dye anions as quenchers of Tb(III)-centred luminescence is conditioned by the energy transfer from $^5D_4$ of Tb(III) to the lower

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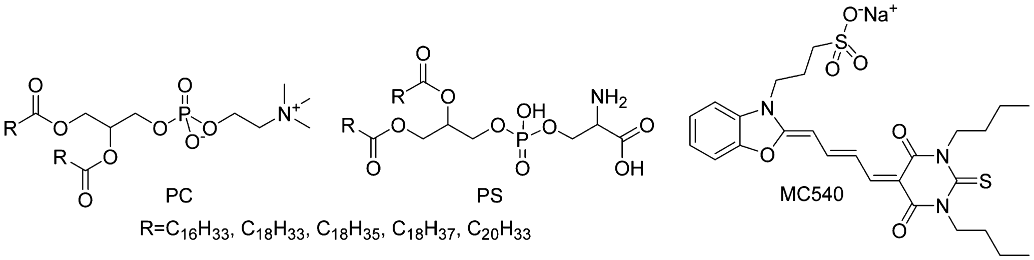

Scheme 1. Schematic structure representation of PC, PS and MC540.

lying triplet levels of dyes [21,22], as well as their affinity towards phospholipid vesicles [25,27]. The choice of Cu(II) ions as inorganic quenchers is inspired by literature data highlighting their binding with PS [28] and the quenching of Tb(III) luminescence via both the energy transfer and the ion exchange [29–32]. The present work introduces the steady state and time resolved Tb(III)-centred luminescence of Tb(III) SNs under various concentration of PC and PS:PC mass ratio in their mixture. The fluorescent results are analyzed in the correlation with dynamic light scattering (DLS), electrokinetic potential and atomic force microscopy (AFM) data.

2. Experimental

2.1. Materials

Tetraethyl orthosilicate (TEOS) 98%, ammonia aqueous solutions (28–30%), n-heptanol 98%, cyclohexane 99%, $CuSO_4{\cdot}5H_2O$ from Acros; terbium(III) nitrate hexahydrate (99.9%) from Alfa Aesar; Tris-(hydroxymethyl)-aminomethane from Scharlau; Triton X-100, merocyanine 540 from Sigma–Aldrich were used as purchased without further purification. Phospholipids, namely L-α-phosphatidylcholine (PC, ≥99%), L-α-phosphatidyl-L-serine (PS, ≥97%) were purchased from Sigma–Aldrich and used as received.

Synthesis of silica coated Tb(III) nanoparticles (42 ± 5 nm) has been performed according to reverse microemulsion procedure presented in the work [33].

2.2. Sample preparation

The vesicle preparation has been performed by the dissolution of PC or PS ($0.4\,g\,L^{-1}$) in bidistilled water filtered through 0.45 μm nylon membrane filter from Millipore with further stirring within half of an hour and sonication for 1 h at 25 °C [34,35]. Both PC and PS exist in liquid crystalline phase at these temperature conditions. The samples for DLS measurements have been prepared by the dilution of the initial vesicle solution by Tris buffer solution (0.1 M) prepared from bidistilled and filtered water with further sonication at 25 °C for 1 h. The interaction of phospholipid vesicles with SNs was induced by the mixture of the definite aliquots of the aqueous dispersion of SNs ($4\,g\,L^{-1}$), vesicle and Tris buffer solutions followed by the stirring of the mixture within half of an hour with further sonication for 1 h at 25 °C.

2.3. Methods

The dynamic light scattering (DLS) measurements were performed by means of the Malvern Mastersize 2000 particle analyzer. A He–Ne laser operating at 633 nm wavelength and emitting vertically polarized light was used as a light source. The measured autocorrelation functions were analyzed by Malvern DTS software and the second-order cumulant expansion methods. The effective hydrodynamic diameter (D) was calculated by the Einstein–Stokes relation from the first cumulant. The average error in these experiments was approximately 4%. All samples were prepared from the bidistilled water filtered through 0.45 μm nylon membrane filter from Millipore.

Zeta potential “Nano-ZS” (MALVERN) using laser Doppler velocimetry and phase analysis light scattering was used for zeta potential measurements.

The steady-state emission spectra were recorded on a spectrofluorometer FL3-221-NIR (Jobin Yvon) under 330 nm.

Luminescence decay measurements were performed using a Horiba Jobin Yvon Fluorolog-3-221 spectrofluorometer with SPEX FL-1042 phosphorimeter accessory using a xenon flash lamp as the photon source with following parameters: time per flash – 50.00 ms, flash count – 200 ms, initial delay – 0.05 ms and sample window – 2 ms. Excitation of samples was performed at 330 nm, and emission detected at 546 nm with 2 nm slit width for both excitation and emission.

UV–vis spectra were recorded on a Lambda 35 spectrophotometer (Perkin-Elmer).

An atomic force microscope (MultiMode V, USA) was used to reveal morphology of the nanoparticles modified by surfactants. The 250–350 kHz canterlivers (Veeco, USA) with silicone tips (tip curvature radius is of 10–13 nm) were used in all measurements. The microscopic images were obtained by means of 8279JV scanner with 256 × 256 resolution. The scanning rate was 1 Hz. Anti-vibrational system (SG0508) was used to eliminate external distortions. The aqueous dispersions of nanoparticles (0.028 and $0.1\,g\,L^{-1}$) were ultrasonicated within 30 min and then the droplet of the sample was placed on mica surface with the roughness no more than 1–5 nm. The AFM imaging was performed after water evaporation.

All samples with nanoparticles were ultrasonicated within 60 min before measurements.

All measurements have been performed at least three times at 25 °C.

The experimental points are the mean of three measurements. The standard deviation for the each experimental point has been calculated on the basis of three (at least) measurements.

3. Results and discussion

3.1. The effect of silica supported PC and mixed PC–PS bilayers on the photophysical properties of the silica coated Tb(III) complexes

The temperature and concentration conditions of the zwitterionic phospholipid bilayers deposition onto silica surface are well documented [25,26]. This deposition is favoured by peculiar conditions (pH, salinity, Tris buffer) [34–39], while the formation of the SLB from the anionic PhLs requires the special pretreatment of SNs by definite anchor groups [17]. In particular the adsorption of cations arisen from Tris buffer at the negatively charged silica surface enhances the deposition of PC. The previously reported Tb(III)-doped silica nanoparticles (SNs) [33] have been applied as silica beads for the adsorption of PC and the mixture of PC and PS.